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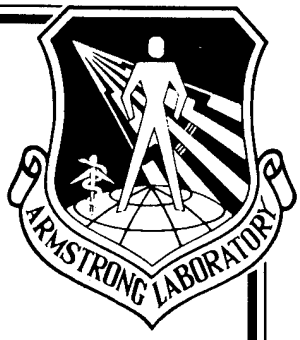

GENETIC TOXICITY EVALUATION OF 1,1,1,2,3,3,3-HEPTAFLUOROPROPANE VOLUME I OF III: RESULTS OF *SALMONELLA TYPHIMURIUM* HISTIDINE REVERSION ASSAY (AMES ASSAY)

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January 1995

FINAL REPORT FOR THE PERIOD MARCH THROUGH DECEMBER 1994

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TECHNICAL REVIEW AND APPROVAL

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VOLUME I

The experiments reported herein were conducted according to the "Guide for the Care and Use of Laboratory Animals," Institute of Laboratory Animal Resources, National Research Council.

This report has been reviewed by the Office of Public Affairs (PA) and is releasable to the National Technical Information Service (NTIS). At NTIS, it will be available to the general public, including foreign nations.

This technical report has been reviewed and is approved for publication.

FOR THE COMMANDER

Terry A. Childress

TERRY A. CHILDRESS, Lt Col, USAF, BSC
Director, Toxicology Division
Armstrong Laboratory

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13. ABSTRACT (Maximum 200 words)

Under subcontract to ManTech Environmental Technology, Incorporated, Genesys Research, Incorporated tested 1,1,12,3,3,3-heptafluoropropane (HFC-227ea) using Billups-Rothenberg exposure chambers for the exposure chamber modification of the *Salmonella typhimurium* histidine (his) reversion mutagenesis system (the Ames test), a microbial assay that measures $his^- \rightarrow his^+$ reversion induced by chemicals that cause base changes or frameshift mutations i the genome of this organism. Testing was conducted using five *Salmonella* strains, with and without metabolic activation.

HFC-227ea was tested in a preliminary test and in a mutagenesis assay. HFC-227ea was tested to toxic levels in the mutagenesis assay, but a sufficient number of nontoxic concentrations were tested to determine if HFC-227ea were capable of inducing a dose-related mutagenic response, and the positive control responses were consistent with historical data from the laboratory, and no evidence of a mutagenic response was obtained in any strain without or with activation. Therefore, HFC-227ea was negative in the *Salmonella typhimurium* histidine reversion mutagenesis test in the presence and absence of metabolic activation.

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PREFACE

The U.S. Air Force is investigating chemical replacements for the fire suppressant/extinguishant Halon 1301. 1,1,1,2,3,3,3-Heptafluoropropane (HFC-227ea) has excellent solvent properties and may serve as a "drop in" extinguishant replacement. Results from laboratory animal in vivo studies indicate that HFC-227ea has a low order of acute toxicity. A comprehensive literature search indicated that no information was available on the mutagenic potential of HFC-227ea. ManTech Environmental initiated a battery of three short-term assays that were utilized to assess the mutagenic and clastogenic potential of HFC-227ea. Protocols for these assays were in conformance with the Environmental Protection Agency's (Toxic Substances Control Act) Health Effects Testing

This document, Volume I of III, serves as a final report detailing the results of the *salmonella typhimurium* histidine reversion assay (Ames assay) in the genetic toxicity evaluation of HFC-227ea. Volumes II and III will describe, respectively, the results of the mouse bone marrow micronucleus test and the results of the forward mutation assay using L5178Y mouse lymphoma cells.

The research described herein began in March 1994 and was completed in December 1994 by Genesys Research, Inc., Research Triangle Park, NC under a subcontract to ManTech Environmental Technology, Inc., Toxic Hazards Research Unit (THRU), and was coordinated by Darol E. Dodd, Ph.D., THRU Laboratory Director. This work was sponsored by the Toxicology Division, Occupational and Environmental Health Directorate, Armstrong Laboratory, and was performed under Department of the Air Force Contract No. F33615-90-C-0532 (Study No. F30). Lt Col Terry A. Childress served as Contract Technical Monitor for the U.S. Air Force, Armstrong Laboratory, Toxicology Division.

SUMMARY

Under subcontract to ManTech Environmental Technology, Incorporated, Genesys Research, Incorporated tested 1,1,1,2,3,3,3-heptafluoropropane (HFC-227ea) using Billups-Rothenberg exposure chambers for the exposure chamber modification of the *Salmonella typhimurium* histidine *(his)* reversion mutagenesis system (the Ames test), a microbial assay that measures his^- —> his^+ reversion induced by chemicals that cause base changes or frameshift mutations in the genome of this organism. Testing was conducted using five *Salmonella* strains, with and without metabolic activation.

HFC-227ea was tested in a preliminary test and in a mutagenesis assay. HFC-227ea was tested to toxic levels in the mutagenesis assay, but a sufficient number of nontoxic concentrations were tested to determine if HFC-227ea were capable of inducing a dose-related mutagenic response, and the positive control responses were consistent with historical data from the laboratory, and no evidence of a mutagenic response was obtained in any strain without or with activation. Therefore, HFC-227ea was negative in the *Salmonella typhimurium* histidine reversion mutagenesis test in the presence and absence of metabolic activation.

GENESYS RESEARCH INCORPORATED'S GOOD LABORATORY PRACTICE COMPLIANCE STATEMENT

MUTAGENESIS TESTING OF 1,1,1,2,3,3,3-HEPTAFLUOROPROPANE (HFC-227ea) USING THE AMES *SALMONELLA TYPHIMURIUM* HISTIDINE REVERSION ASSAY FOR VOLATILE CHEMICALS, WITH AND WITHOUT METABOLIC ACTIVATION

Genesys Research Incorporated's portion of the above titled study was reviewed for compliance with Quality Assurance (QA) regulations and with the provisions of the United States Environmental Protection Agency/Toxic Substances Control Act Good Laboratory Practice (GLP) Standards as defined in the Federal Register, August 17, 1989 (40 CFR, Part 792) and TSCA Test Guidelines, Federal Register, September 27, 1985 (Vol. 50, #188, Part 798.5265) and its revision (May 20, 1987, Vol. 52, #97).

The practices used in the study were found to be in compliance with these regulations.

Ann D. Mitchell 12/17/94

Ann D. Mitchell, Ph.D. Date
Study Director

GENESYS RESEARCH INCORPORATED'S QUALITY ASSURANCE STATEMENT

With the exception of the handling, storage, dilution (for exposure of the bacteria) and analytical chemistry of the test material, which were the responsibility of ManTech Environmental Technology, Incorporated, the data and the report for the following study carried out at Genesys Research, Incorporated has been reviewed and approved for compliance with the provisions of the United States Environmental Protection Agency/Toxic Substances Control Act Good Laboratory Practice (GLP) Standards as defined in the Federal Register, August 17, 1989 (40 CFR, Part 792) and TSCA Test Guidelines, Federal Register, September 27, 1985 (Vol. 50, #188, Part 798.5265) and its revision (May 20, 1987, Vol. 52, #97).

The final report accurately describes the methods that were used and accurately reflects the raw data of the study.

ManTech Environmental Technology Incorporated Study Number: 1093-F30

Genesys Research, Incorporated Study Number: 94035

Type Study: *Salmonella typhimurium* histidine reversion assay

Protocol Signed by Study Director: March 19, 1994

Date Testing Started: March 29, 1994

Critical Phase Audit(s): March 29 and May 25, 1994

Date Testing Completed: May 25, 1994

Date Draft Report Audited: August 26, 28 and 29, 1994

Date Audit Findings Reported to Management: March 29, May 25 and September 9, 1994

Approved: Helen M. King
Helen M. King, B.S.
Quality Assurance Officer for Genesys

Date: 12/17/94

MANTECH ENVIRONMENTAL TECHNOLOGY, INCORPORATED GOOD LABORATORY PRACTICE COMPLIANCE STATEMENT

Study Title: *In Vitro* and Inhalation Toxicity Study of 1,1,1,2,3,3,3-Heptafluoropropane

Project Number: 1093-F30

Study Director: Allen Ledbetter

ManTech Environmental Technology's portion of this study was conducted in accordance with EPA Good Laboratory Practice Regulations (GLP) as set forth in the Code of Federal Regulations (40 CFR 792). There were no significant deviations, in the work conducted by ManTech, from the aforementioned GLP regulations that would have affected the integrity of the study or the interpretation of the test results. The ManTech generated raw data have been reviewed by the Study Director, who certifies that the information contained in this report represents an appropriate and accurate conclusion within the context of the study design and evaluation criteria. Deviations are listed below:

1. The sponsor was responsible for the test substance characterization, stability and homogeneity analysis.

All original ManTech generated raw data are retained in the ManTech Environmental Technology's Archives, at 5 Triangle Drive, Research Triangle Park, NC 27709, with a copy of the final study report.

SUBMITTED BY:

Study Director:

Allen Ledbetter 12/16/94

Allen Ledbetter Date

MANTECH ENVIRONMENTAL TECHNOLOGY, INCORPORATED QUALITY ASSURANCE STATEMENT

Study Title: *In Vitro* and Inhalation Toxicity Study of 1,1,1,2,3,3,3-Heptafluoropropane

Project Number: 1093-F30

Study Director: Allen Ledbetter

Report Audit Dates:

This study has been subjected to inspections and the report has been audited by ManTech Environmental Technology's Quality Assurance Unit. The report describes the methods and procedures used in the study and the reported results accurately reflect ManTech's raw data. ManTech's raw data and a copy of the final report will be stored in room 210 in the MET building at Research Triangle Park, NC. The sponsor was responsible for the Iodotrifluoromethane characterization, stability and homogeneity analyses.

The following are the inspection dates, and the dates inspection reports were submitted:

Phase(s)	Date(s) of Inspection	Report Submitted to Study Director	Report Submitted to Management
Protocol	8/2/94	8/2/94	8/2/94
Data Review	12/15/94	12/16/94	12/16/94

12/16/94

Terry F. Walser Date
Quality Assurance Officer

MUTAGENESIS TESTING OF 1,1,1,2,3,3,3-HEPTAFLUOROPROPANE (HFC-227ea) USING THE AMES *SALMONELLA TYPHIMURIUM* HISTIDINE REVERSION ASSAY FOR VOLATILE CHEMICALS, WITH AND WITHOUT METABOLIC ACTIVATION

1. INTRODUCTION

Under subcontract to ManTech Environmental Technology, Incorporated (ManTech), Dayton, Ohio (ManTech/Dayton) Genesys Research, Incorporated (Genesys) used the exposure chamber modification of the *Salmonella typhimurium* histidine *(his)* reversion mutagenesis assay to examine the potential of 1,1,1,2,3,3,3-heptafluoropropane (HFC-227ea) to induce frame shift and base pair substitution *his*$^-$ —> *his*$^+$ reversion mutations. Allen Ledbetter, ManTech Environmental Technology, Incorporated, Research Triangle Park, North Carolina (ManTech/RTP), was responsible for handling, storage, dilution (for exposure of the bacteria), and analytical chemistry of the test material.

Testing at Genesys consisted of all procedures not performed by ManTech/RTP and was conducted under the direction of Ann D. Mitchell, Ph.D., Study Director, by J. Thom Deahl, M.S., and Diane M. Brecha, B.S., Genetic Toxicologists, in accordance with the provisions of the United States Environmental Protection Agency/Toxic Substances Control Act Good Laboratory Practice (GLP) Standards as defined in the Federal Register, August 17, 1989 (40 CFR, Part 792) and TSCA Test Guidelines, Federal Register, September 27, 1985 (Vol. 50, #188, Part 798.5265) and its revision (May 20, 1987, Vol. 52, #97). Testing for this study was initiated with a preliminary concentration range-finding assay on March 29, 1994 and concluded on May 25, 1994 with mutagenesis assay plate counts. The protocol, a protocol amendment, raw data obtained by Genesys, and a copy of this report will be retained in Genesys' archives located at 2300 Englert Drive, Durham, NC 27713.

2. BACKGROUND

Evidence suggests that a high percentage of chemicals that elicit a mutagenic response in the *Salmonella* assay are potential animal and human mutagens and carcinogens (McCann et al., 1975a; McCann and Ames, 1976; Sugimura et al., 1976; Tennant et al., 1987). Because the *Salmonella* assay is efficient, can indicate mechanisms of chemical interaction with DNA, and produces few positive results for noncarcinogens, it is the cornerstone of evaluations for genotoxicity.

The *Salmonella typhimurium* strains used for the reverse mutation assay are histidine auxotrophs by virtue of mutations in the histidine operon. When these histidine-dependent cells are grown on minimal medium agar plates containing a trace of histidine, only those cells that revert to histidine independence are able to form colonies. The small amount of histidine allows all the plated bacteria to undergo a few divisions; in many cases, this growth is essential for mutagenesis to occur. The histidine rever-

tants are easily visible as colonies against the slight background growth. The spontaneous reversion rate of each strain is relatively constant (Maron and Ames, 1983; McCann et al., 1975a), but when a mutagen is added to the agar, the mutation rate is increased, usually in a dose-related manner.

Strains TA1535 and TA100 are reverted to histidine independence by many mutagens that cause base-pair substitutions; strains TA1537, TA1538 and TA98 are reverted by many frameshift mutagens. In addition to having mutations in the histidine operon, all the indicator strains have a mutation (*rfa*) that leads to a defective lipopolysaccharide coat; they also have a deletion that covers genes involved in the synthesis of the vitamin biotin (*bio*) and in the repair of ultraviolet (uv)-induced DNA damage (*uvrB*). The *rfa* mutation makes the strains more permeable to many large molecules. The *uvrB* mutation renders the bacteria unable to use the accurate excision repair mechanism to remove certain chemically or physically damaged DNA and thereby enhances the strain's sensitivity to some mutagenic agents.

Strains TA100 and TA98 are derived from strains TA1535 and TA1538, respectively, and contain the resistance transfer factor, plasmid pKM101, which is believed to cause an additional increase in error-prone DNA repair, leading to even greater sensitivity to most mutagens (Ames et al., 1975, Maron and Ames, 1983, McCann et al., 1975b). In addition, plasmid pKM101 confers resistance to the antibiotic ampicillin, which is a convenient marker for detecting the presence of plasmid pKM101 in these strains.

In the standard plate incorporation *Salmonella* histidine reversion assay, the test material, bacteria, and either a metabolic activation mixture (S9) or buffer are added to liquid top agar which is mixed then immediately poured on a plate of bottom agar. However, volatile, water-insoluble compounds may be poorly detected in the standard plate incorporation assay and may be tested, instead, with a preincubation modification (Maron and Ames, 1983). For this approach, the test material, bacteria, and S9 (if used) are incubated for 20-30 min. at 37°C, then top agar is added, and the mixture is poured on a plate of bottom agar. Increased activity with preincubation is attributed to the fact that the test compound, bacteria, and S9 are incubated at higher concentrations (without agar present) than in the standard plate incorporation test.

In contrast to the plate incorporation assay and the preincubation modification, test materials which are volatile at ambient temperature are tested by the exposure chamber modification (Zeiger *et al.*, 1992). In this procedure, the *Salmonella* strains and S9 mix, or buffer, are incorporated into the top agar then poured onto the minimal agar plates, the plates are stacked in an exposure chamber, and the air is evacuated. A measured amount of the test gas is introduced and air is allowed to flow in until atmospheric pressure is reached. The chamber is then sealed and placed at 37°C for 48 hr. At the end of this period, the exposure chamber is removed to a 100%-exhausted laminar flow hood, the lid removed, and the residual test gas allowed to dissipate for at least 20-30 minutes. The plates are then removed from the exposure chamber, and the colonies are counted.

Because HFC-227ea is a volatile material, it was tested by the exposure chamber modification, and plastic Billups-Rothenberg exposure chambers were used for the exposure periods.

3. METHODS

3.1. Identification, Storage, and Dilution of the Test Material

The test material, 1,1,1,2,3,3,3-heptafluoropropane (HFC-227ea, or F_7C_3H; molecular weight 170; CAS Number 431-89-0), a colorless gas, was received in a steel gas container from ManTech/Dayton on April 7, 1994 then transferred to Allen Ledbetter, ManTech/RTP, who was responsible for handling, storage, and dilution of the test material. The HFC-227ea was stored at ManTech/RTP at room temperature (approximately 72°F). ManTech/Dayton documented the strength, purity, and composition of the test material and provided a Material Safety and Data Sheet (MSDS) from Great Lakes Chemical Corporation for HFC-227ea. Upon acceptance of the final report, the remaining test material will be returned to the Sponsor. No reserve sample will be retained by ManTech/RTP.

3.2. Tester Strains

The tester strains used in this study were TA1535, TA1537, TA1538, TA98, and TA100, obtained from Dr. Bruce Ames of the University of California at Berkeley (Ames *et al.*, 1975). These indicator strains are kept frozen (-80°C) in nutrient broth supplemented with 8.3% sterile glycerol in 1.8-ml aliquots containing about 4×10^8 cells/ml that had been checked for their genotypic characteristics (*his, rfa, uvrB, bio*) and for the presence of the plasmid. Before each experiment, the cultures were grown overnight with shaking at 37°C in Oxoid No. 2 broth.

3.3. Metabolic Activation System

Rat liver S9 homogenate, in KCl buffer, prepared aseptically from Aroclor 1254-induced male Sprague-Dawley rats, was obtained from Molecular Toxicology, Inc., Annapolis, MD, and stored frozen in liquid nitrogen. The sterile S9 was thawed and used to prepare an S9 mixture immediately before the chemical exposure step of each assay by addition of the homogenate to a mixture of cofactors sterilized by filtration. Therefore, no filtration of the S9 homogenate or S9 mixture was required. The final concentrations of the S9 mixture were 50 µl/ml S9 homogenate, 4 µmol/ml NADP, 5 µmol/ml D-glucose-6-phosphate, 8 µmol/ml $MgCl_2$, 33 µmol/ml KCl, and 100 µmol/ml sodium phosphate buffer (pH 7.4).

3.4. Controls

Concurrent sterility, negative, and positive controls were used in each assay. Sterility controls included separately plating out the top agar, metabolic activation mixture, and buffer. For negative controls, plates containing bacteria, top agar and buffer or, for tests

with metabolic activation, the metabolic activation mixture, were exposed to filtered air.

The following positive control chemicals were used: sodium azide (CAS No. 26628-22-8) for the base-pair substitution mutants TA1535 and TA100; 9-aminoacridine (CAS No. 90-45-9) for the frameshift mutant TA1537; 4-nitro-*o*-phenylenediamine (CAS No. 99-56-9) for the frameshift mutants TA98 and TA1538; and 2-anthramine (CAS No. 613-13-8) for all tester strains in the presence of metabolic activation.

3.5. General Procedure

The specific test method has been described in detail (Ames et al., 1975; Maron and Ames, 1983). The exposure chamber assay for each sample was performed in the following way. To a sterile glass test tube was added: 0.1 ml of indicator organisms (about 10^8 bacteria), 0.5 ml of the metabolic activation mixture or buffer, and 2.00 ml of molten 0.615% agar. In addition, 0.1 ml of the appropriate positive control was added to each positive control plate. This top agar mixture was then vortexed gently and poured onto a prelabeled plate containing about 25 ml of minimal glucose agar plus biotin.

After the top agar had set, the test material (or negative control) plates for all five strains that were to be exposed to one concentration of the test material (or to air, the negative control) were placed on a shelf on the bottom section of plastic exposure chamber (Modular Incubator Chamber, Billups-Rothenberg, Del Mar, CA) with an internal volume of approximately 1 liter. The chambers, which consisted of an upper and lower section connected by a gasket and a large stainless steel adjustable squeeze clamp, were then closed by connecting and sealing the upper and lower sections.

The exposure chambers were transported from Genesys to ManTech/RTP where they were labeled as to the desired concentrations; then gas was introduced into an inlet port in the bottom section of each chamber, circulated throughout the chamber, and drawn out of an exhaust port, which was also located in the bottom section. (See Exposure Method, below.) Chamber atmosphere samples, for infrared (IR) analysis were collected from the exhaust port.

The chambers containing the plates were then returned to Genesys where they were incubated at 37°C for approximately 48 hours. Exposure chambers were not required for plates of bacteria treated with positive controls, which were also incubated at 37°C for approximately 48 hours. Following the exposure period, the plates were removed from the exposure chambers, and the number of histidine independent revertant colonies on each plate was counted using an Artek 982B colony counter, or by hand if the colonies could not be counted accurately with the Artek.

3.6. Exposure Method

The test material and dilution air were metered through calibrated flowmeters into the exposure chambers, and chamber atmosphere samples were collected, via a gas tight syringe, and injected into the IR instrument for analysis. The test material and the

dilution air were adjusted until the desired chamber concentration was obtained. The chamber exhaust was first disconnected, and then the gases were shut off. This was done to prevent diluting the chamber atmosphere. The inlet and exhaust ports were then sealed with screw clamps. During the mutagenesis assay, two chambers per exposure level were required to hold all the petri plates. To ensure that both chambers received the same concentration, the gas was introduced to the inlet port of one chamber which was exhausted into the inlet of the second chamber. The exhaust from the second chamber was then analyzed by IR.

3.7. Analysis of Chamber Atmospheres

Prior to analyzing the chamber atmospheres, the IR instrument (Miran 1A, Foxboro Corp., Foxboro, MA) was calibrated using a "closed-loop" method. IR instrument operating parameters were: wavelength, 9.7 microns; pathlength, 6.75 meters; absorbance, 0.25; slit, 1; and range, X1. A calibration curve was prepared using concentration versus recorder chart lines. A Texas TI-60 calculator (Texas Instruments, Lubbock, TX) was used to determine the calibration curve using a least-squares method. The chamber atmospheres were then analyzed by withdrawing a volume of the atmosphere with a gas-tight syringe and injecting it into the IR instrument which was in the "closed-loop" configuration. The number of chart lines was entered into the calculator and the corresponding concentration obtained. The concentration was then corrected for the injection volume.

3.8. Preliminary Range-Finding Assay

A preliminary assay of HFC-227ea was conducted with strain TA100 in the presence and absence of metabolic activation to determine suitable nontoxic dose ranges for the mutagenicity assays. The results of each testing condition, without and with activation, were evaluated separately. Toxic concentrations were defined as those that produced a decrease in the number of colonies, or a clearing in the density of the background lawn, or both.

3.9. Mutagenesis Assay

Once a dose range had been established, HFC-227ea was assayed utilizing the five tester strains (TA1535, TA1537, TA1538, TA98, and TA100) over 5 dilutions of the test material such that the highest concentration would be one expected to result in toxicity or would be the highest concentration that could be tested. The mutagenesis assay were conducted using three plates per dose level, in the presence and absence of S-9 metabolic activation. The procedures used were the same as in the preliminary assay except that, because 30 plates were exposed to each test material concentration (5 strains x 3 plates/concentration x 2 [without and with activation]), two chambers, connected in series, were used to hold the 30 plates per concentration.

3.10. Raw Data Collection

All observations, raw data collected, and calculations were recorded onto standard forms which were bound together with the study protocol at the conclusion of testing.

The actual number of revertant colonies was reported for each plate, and the mean number of revertant colonies per plate and the standard deviation were reported for each concentration of each test material and for the positive and negative controls. Concentrations that resulted in a mutagenic response were indicated with a ♦.

3.11. Analysis and Interpretation of Results

The data generated were considered acceptable if the controls were within the laboratory's historical ranges and if a sufficient number of nontoxic concentrations were tested to determine if a test material were capable of inducing a dose-related mutagenic response.

A test material is considered mutagenic for a condition and strain if a dose-related increase in the number of revertants is observed over three concentrations and the highest increase in strains TA1535, TA1537 and TA1538 is equal to three times the solvent control value or the highest increase in strains TA98 and TA100 is equal to two times the solvent control value (Brusick in Hayes, 1989). A test material is considered negative if the criteria for a positive response are not met and the positive control values are within the historical range for the laboratory.

Both biological and statistical significance were considered together in the evaluation of the results; the final interpretation of the results was the responsibility of the Study Director.

4. RESULTS AND DISCUSSION

The analysis of the exposure chamber atmospheres indicated that the desired concentrations were achieved within 10%, as summarized in Table 1.

The results obtained for testing HFC-227ea in the *Salmonella typhimurium* histidine reversion exposure chamber assays are presented in Tables 2 and 3. All positive and negative control values were within the laboratory's appropriate ranges, which are consistent with the ranges reported in the literature, and a sufficient number of nontoxic concentrations were tested in the mutagenesis assay; therefore, the data were acceptable.

In the preliminary assay, Table 2, HFC-227ea was tested in TA100 over 5 concentrations ranging from 104,730 to 835,390 ppm HFC-227ea, and no toxicity or mutagenic response was observed. In the mutagenesis assay, Table 3, HFC-227ea was tested in the five tester strains over 4 concentrations ranging from 438,707 to 934,548 ppm HFC-227ea, the highest concentration that could be obtained under the conditions of testing. In this assay, toxicity, as indicated by a decrease in the number of colonies at the highest concentra-

tions tested, was obtained for all five strains in the absence and presence of activation. In addition, numerous tiny colonies were present at the highest concentrations tested under both testing conditions for all strains except TA1537 in the presence of activation. The tiny colonies were further evidence of toxicity, as they arise from the background lawn when the number of bacteria is reduced such that sufficient histidine is available for additional growth of the nonmutated bacteria that form the background lawn.

HFC-227ea was tested to toxic levels in the mutagenesis assay, a sufficient number of nontoxic concentrations were tested to determine if HFC-227ea were capable of inducing a dose-related mutagenic response, and the positive control responses were consistent with historical data from the laboratory. But no evidence of a mutagenic response was obtained in any strain without or with activation. Therefore, HFC-227ea was negative in the *Salmonella typhimurium* histidine reversion mutagenesis test in the presence and absence of metabolic activation.

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Table 1

COMPARISON OF NOMINAL AND IR DETERMINED CONCENTRATIONS OF 1,1,1,2,3,3,3-HEPTAFLUOROPROPANE (HFC-227ea) IN THE *SALMONELLA TYPHIMURIUM* ASSAYS

	Desired Concentration(ppm)	IR Determined Concentration (ppm)	% of Desired
Preliminary Assay	100,000	104,730	104.7
	200,000	184,414	92.2
	500,000	531,970	106.4
	700,000	768,139	109.7
	900,000	835,930	92.9
Mutagenesis Assay	450,000	438,707	97.5
	600,000	598,432	99.7
	750,000	772,997	103.1
	900,000	934,548	103.8

Table 2

PRELIMINARY *SALMONELLA TYPHIMURIUM* ASSAY OF 1,1,1,2,3,3,3-HEPTAFLUOROPROPANE (HFC-227ea)

Chemical	S9	Dose Per Plate	TA100 Histidine Revertant Colonies/Plate			Mean ± S.D.	Notes
Air	-	N/A	91	171	96	119 ± 29.4	
			122	130	106		
HFC-227ea	-	104,730 ppm	73	135	106	105 ± 31.0	
	-	184,414 ppm	93	70	117	93 ± 23.5	
	-	598,432 ppm	130	145	104	126 ± 20.7	
	-	768,130 ppm	166	93	109	123 ± 38.4	
	-	835,390 ppm	99	109	130	113 ± 15.8	4+
Sodium azide	-	1.5 µg	1,122	1,621	1,632	1,458 ± 291.3	◆
Air	+	N/A	143	145	125	148 ± 18.6	
			179	158	138		
HFC-227ea	+	104,730 ppm	197	200	119	172 ± 45.9	
	+	184,414 ppm	132	N	93	113 ± 27.6	
	+	598,432 ppm	177	135	119	144 ± 30.0	
	+	768,130 ppm	171	117	112	133 ± 32.7	
	+	835,390 ppm	143	N	122	133 ± 14.8	4+
2-Anthramine	+	2.5 µg	858	2,070	499	1,142 ± 823.2	◆

4+ = Normal background lawn
◆ = Positive
N = No data

Table 3

SALMONELLA TYPHIMURIUM MUTAGENESIS ASSAY OF 1,1,1,2,3,3,3-HEPTAFLUOROPROPANE (HFC-227ea)

Chemical	S9	Dose Per Plate	Strain	Histidine Revertant Colonies/Plate			Mean	±	S.D.	Notes
Air	-	N/A	TA1535	10	14	11	13	±	2.2	
				14	14	16				
HFC-227ea	-	438,707 ppm	TA1535	22	29	20	24	±	4.7	
	-	598,432 ppm	TA1535	25	15	22	21	±	5.1	
	-	772,997 ppm	TA1535	27	20	18	22	±	4.7	
	-	934,548 ppm	TA1535	5	7	0	6	±	3.6	T
Sodium azide	-	1.5 µg	TA1535	772	904	872	849	±	68.9	◆
Air	+	N/A	TA1535	13	8	5	11	±	4.8	
				19	9	11				
HFC-227ea	+	438,707 ppm	TA1535	20	19	18	19	±	1.0	
	+	598,432 ppm	TA1535	19	19	13	17	±	3.5	
	+	772,997 ppm	TA1535	13	7	21	14	±	7.0	
	+	934,548 ppm	TA1535	6	10	9	8	±	2.1	T
2-Anthramine	+	2.5 µg	TA1535	544	456	436	479	±	57.5	◆
Air	-	N/A	TA1537	7	8	3	5	±	2.7	
				5	7	1				
HFC-227ea	-	438,707 ppm	TA1537	5	7	4	5	±	1.5	
	-	598,432 ppm	TA1537	5	5	6	5	±	0.6	
	-	772,997 ppm	TA1537	1	3	2	2	±	1.0	
	-	934,548 ppm	TA1537	2	0	1	1	±	1.0	T
9-AA	-	100.0 µg	TA1537	1,520	2,024	1,248	1,597	±	393.7	◆
Air	+	N/A	TA1537	7	7	7	8	±	1.0	
				8	9	9				
HFC-227ea	+	438,707 ppm	TA1537	5	4	5	5	±	0.6	
	+	598,432 ppm	TA1537	10	7	3	7	±	3.5	
	+	772,997 ppm	TA1537	4	9	4	6	±	2.9	
	+	934,548 ppm	TA1537	2	2	1	2	±	0.6	4+
2-Anthramine	+	2.5 µg	TA1537	272	192	188	217	±	47.4	◆

Table 3 (continued)

Chemical	S9	Dose Per Plate	Strain	Histidine Revertant Colonies/Plate			Mean	±	S.D.	Notes
Air	-	N/A	TA1538	15	8	12	13	±	4.1	
				19	9	14				
HFC-227ea	-	438,707 ppm	TA1538	17	15	15	16	±	1.2	
	-	598,432 ppm	TA1538	21	22	13	19	±	4.9	
	-	772,997 ppm	TA1538	3	2	4	3	±	1.0	
	-	934,548 ppm	TA1538	4	5	2	4	±	1.5	T
4-NOPD	-	2.0 µg	TA1538	304	260	292	285	±	22.7	◆
Air	+	N/A	TA1538	17	14	21	16	±	3.1	
				17	16	12				
HFC-227ea	+	438,707 ppm	TA1538	20	18	18	19	±	1.2	
	+	598,432 ppm	TA1538	21	16	19	19	±	2.5	
	+	772,997 ppm	TA1538	12	19	15	15	±	3.5	
	+	934,548 ppm	TA1538	3	2	6	4	±	2.1	T
2-Anthramine	+	2.5 µg	TA1538	972	1,288	1,584	1,281	±	306.1	◆
Air	-	N/A	TA98	17	22	13	17	±	4.8	
				15	22	10				
HFC-227ea	-	438,707 ppm	TA98	19	14	14	16	±	2.9	
	-	598,432 ppm	TA98	18	17	19	18	±	1.0	
	-	772,997 ppm	TA98	4	4	4	4	±	0.0	T
	-	934,548 ppm	TA98	1	6	2	3	±	2.6	T
4-NOPD	-	2.0 µg	TA98	396	408	364	389	±	22.7	◆
Air	+	N/A	TA98	24	17	18	22	±	4.9	
				23	30	19				
HFC-227ea	+	438,707 ppm	TA98	19	28	25	24	±	4.6	
	+	598,432 ppm	TA98	10	9	21	13	±	6.7	T
	+	772,997 ppm	TA98	12	7	5	8	±	3.6	T
	+	934,548 ppm	TA98	2	8	10	7	±	4.2	T
2-Anthramine	+	2.5 µg	TA98	1,528	1,544	1,224	1,432	±	180.3	◆

Table 3 (concluded)

Chemical	S9	Dose Per Plate	Strain	Histidine Revertant Colonies/Plate			Mean	±	S.D.	Notes
Air	-	N/A	TA100	79	81	76	74	±	8.6	
				58	80	72				
HFC-227ea	-	438,707 ppm	TA100	74	83	91	83	±	8.5	
	-	598,432 ppm	TA100	83	83	97	88	±	8.1	
	-	772,997 ppm	TA100	55	71	81	69	±	13.1	
	-	934,548 ppm	TA100	11	32	56	33	±	22.5	T
Sodium azide	-	1.5 µg	TA100	1,040	704	1,008	917	±	185.4	◆
Air	+	N/A	TA100	84	99	109	92	±	10.3	
				86	89	83				
HFC-227ea	+	438,707 ppm	TA100	80	87	101	89	±	10.7	
	+	598,432 ppm	TA100	87	94	95	92	±	4.4	
	+	772,997 ppm	TA100	73	69	84	75	±	7.8	
	+	934,548 ppm	TA100	45	65	69	60	±	12.9	T
2-Anthramine	+	2.5 µg	TA100	1,920	1,328	1,192	1,480	±	387.1	◆

4NOPD = 4-Nitro-*o*-phenylenediamine
9-AA = 9-Aminoacridine
◆ = Positive

4+ = Normal background lawn
T=Toxicity indicated by the presence of tiny colonies (not included in colony counts)

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